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RESEARCH STUDENT	**Ms. MOHINI SANJAY MANE**
MAIL ID.	manemohini77@gmail.com
MOBILE NO.	**8796162561**
CATEGORY	OBC
SUBJECT	PHARMACEUTICAL SCIENCES & TECHNOLOGY
Ph.D. SYNOPSIS TITLE	Assessment of Anti-Obesogenic and Anti-Dyslipidemic / Anti-Atherosclerotic Potential of Selected Phytoconstituents/Extract of Spices used in Indian Cuisine: Employing Network Pharmacology, Docking, *In Vitro* and *In Vivo* Methods
RESEARCH GUIDE	**Dr. DHAWALE SHASHIKANT C.**
MAIL ID.	shashiprathmesh@gmail.com
MOBILE NO.	**09970700030 / 07020625083**
DESIGNATION	SENIOR PROFESSOR , DEPT. OF PHARMACOLOGY, SCHOOL OF PHARMACY, S.R.T.M. UNIVERSITY, NANDED.
RESEARCH CENTRE	SCHOOL OF PHARMACY, S.R.T.M.UNIVERSITY, NANDED.
UNIVERSITY	SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED-431606, (MAHARASHTRA STATE), INDIA.
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Assessment of Anti-Obesogenic and Anti-Dyslipidemic / Atherosclerotic Potential of Selected Phytoconstituents/Extract of Spices used in Indian Cuisine: Employing Network Pharmacology, Docking, *In Vitro* and *In Vivo* Methods

A SYNOPSIS SUBMITTED FOR THE REGISTRATION OF *DEGREE OF*

DOCTOR OF PHILOSOPHY

In the Faculty of Pharmaceutical Sciences & Technology

Submitted by

Ms. MOHINI SANJAY MANE
M. Pharm.

Under the Guidance of

Dr. DHAWLE SHASHIKANT C. M. Pharm., Ph.D.
Senior Professor,
SCHOOL OF PHARMACY,
S.R.T.M. UNIVERSITY, NANDED

स्वामी रामानंद तीर्थ
मराठवाडा विद्यापीठ,नांदेड.

Swami Ramanand Teerth Marathwada University,

Nanded-431606, (Maharashtra State), India.

(2024-2025)

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ABSTRACT OF PROPOSED WORK PLAN

The present study aims to assess the anti-Obesogenic and anti-Dyslipidemic / anti-Atherosclerotic potential of selected phytoconstituents derived from Indian spices, employing a multidisciplinary approach involving network pharmacology, molecular docking, and *in vitro* and *in vivo* experimental methods. Indian spices such as cinnamon, ginger, black pepper, and turmeric are rich in bioactive compounds like cinnamaldehyde, gingerol, piperine, and curcumin, respectively, which have shown promise in managing metabolic disorders.

The research will begin with the collection of selected spices during their optimal harvesting seasons, followed by authentication, cleaning, drying, and extraction using solvents of varying polarity. Preliminary phytochemical screening will be performed to identify major phytoconstituents. Subsequently, network pharmacology approaches will be employed to predict the molecular targets and biological pathways associated with obesity, atherosclerosis, and dyslipidemia. Protein-ligand interactions will be evaluated using molecular docking studies to identify the binding affinities of key phytoconstituents with relevant target proteins.

The extracts or isolated compounds will then be evaluated in vitro using cell lines to study their effects on adipogenesis, lipid metabolism, and inflammation. For in vivo studies, animal models of obesity, atherosclerosis, and dyslipidemia will be induced through high-fat diets or other established methods. Parameters such as body weight, lipid profiles, inflammatory markers, and histopathological changes will be assessed.

The data will be statistically analyzed to confirm the efficacy of the phytoconstituents in modulating metabolic pathways. The findings from this study are expected to contribute to the development of spice-based therapeutic interventions for the prevention and management of obesity, atherosclerosis, and dyslipidemia, offering safer and natural alternatives for healthcare.

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INTRODUCTION

1) International Status (Worldwide)

Atherosclerosis and obesity are two of the most pressing global health challenges, with significant impacts on morbidity and mortality. According to the World Health Organization (WHO), cardiovascular diseases (CVDs), a major consequence of atherosclerosis, account for 17.9 million deaths annually, making them the leading cause of death worldwide(WHO., www.who.com 2022). Cardiovascular diseases. Simultaneously, obesity has reached epidemic proportions, with more than 1.9 billion adults classified as overweight and over 650 million categorized as obese in 2022. This dual epidemic contributes to increased risks of type 2 diabetes, metabolic syndrome, and CVDs, underscoring the urgent need for effective therapeutic strategies. Phytochemicals derived from medicinal plants have shown promise as alternatives to synthetic drugs. Plants such as Cinnamon (Cinnamomum verum), Black Pepper (Piper nigrum), Ginger (Zingiber officinale), and Curcumin (active compound from Curcuma longa) are rich sources of bioactive compounds, including polyphenols, flavonoids, and alkaloids. These phytochemicals exhibit anti-inflammatory, antioxidant, and lipid-lowering effects, which are crucial for combating atherosclerosis and obesity. Recent studies have shown that curcumin, for example, can modulate oxidative stress and inflammation by targeting the NF-κB pathway, while gingerol from ginger exhibits lipid-lowering properties by enhancing peroxisome proliferator-activated receptor (PPAR) signaling. These findings indicate the therapeutic potential of natural compounds in managing metabolic and cardiovascular disorders (Al-Ishaq *et al.*, 2021). Despite the promising findings of *in vitro* studies, the translation of these results to clinical settings remains a challenge. There is a critical need for systematic *in vivo* studies to validate the efficacy and safety of these phytoconstituents. By leveraging advancements in bioinformatics and pharmacokinetics, researchers can optimize the use of these natural compounds as viable therapeutic agents.

2) National Status (In India)

India is facing a growing burden of non-communicable diseases, with obesity and atherosclerosis among the most significant contributors to mortality and healthcare costs. According to the Indian Council of Medical Research (ICMR), obesity affects nearly 22% of the urban population, with a notable rise in rural regions as well. Concurrently, CVDs are responsible for 28% of deaths annually in India, driven primarily by atherosclerosis. Rapid urbanization, sedentary lifestyles, and changes in dietary patterns have exacerbated these trends. India's traditional medicine systems, such as Ayurveda, have long utilized medicinal plants for health management (Thakkar *et al.*, 2020).. Species like Cinnamon, Black Pepper, Ginger, and Turmeric (Curcuma longa) are integral to Indian diets and therapeutic practices. These plants contain bioactive compounds such as cinnamaldehyde, piperine,

gingerol, and curcumin, which have

been shown to regulate lipid metabolism, reduce oxidative stress, and inhibit inflammatory pathways. For instance, piperine enhances the bioavailability of curcumin, making their combination particularly effective in therapeutic formulations (Patel *et al.* 2020).Despite this rich ethnobotanical heritage, the integration of these plants into modern therapeutic strategies remains limited. There is an urgent need to validate their traditional uses through scientific research, focusing on in vivo and clinical evaluations. Such efforts could pave the way for cost-effective, accessible, and culturally relevant solutions to India's escalating health challenges (OECD, 2020).

3) Significance of the Study

This study aims to evaluate the biological efficacy of phytoconstituents of selected spices in managing atherosclerosis and obesity, focusing on their phytochemical properties and mechanisms of action. The significance of this research lies in several key areas: The exploration of natural compounds as potential therapeutics provides a safer and more holistic alternative to synthetic drugs, which often carry risks of side effects and long-term complications. For instance, while statins are effective in managing hypercholesterolemia, they can lead to myopathy and an increased risk of diabetes with prolonged use. In contrast, natural compounds like curcumin and piperine have demonstrated comparable efficacy in reducing cholesterol levels without significant adverse effects. The study also emphasizes the importance of systemic and *in vivo* evaluations to bridge the gap between preclinical findings and clinical applications. By investigating the pharmacokinetics, bioavailability, and dose-dependent effects of these phytochemicals in experimental models, this research seeks to provide a robust evidence base for their therapeutic use. Moreover, the research highlights the economic and cultural relevance of medicinal plants. Given their widespread availability and affordability, plant-based therapies hold immense potential for reducing healthcare costs and improving access to treatment, particularly in low- and middle-income countries. This aligns with global health goals to promote sustainable and equitable healthcare solutions.

REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT

The study evaluated the aqueous extracts of cinnamon and clove for their anti-atherosclerotic and hypolipidemic activity using hypercholesterolemic zebrafish. The extracts displayed potent inhibition of protein glycation, copper-mediated LDL oxidation, and phagocytosis by macrophages. *In vivo* results showed that clove extract reduced serum cholesterol and triglycerides by 68% and 80%, respectively, while exhibiting antioxidant properties and mitigating body weight gain during a high-cholesterol diet.

Extraction involved 1 g of herbs and spices in water (10 mL) at ambient temperature for 2 hours (Jin and Cho, 2011). The study traced the medicinal pathways of flaxseed (Linum usitatissimum) using bioinformatics tools. Their findings highlighted flaxseed's therapeutic potential for diabetes, cardiovascular diseases (CVDs), and inflammatory bowel diseases. Key target proteins and molecular mechanisms were explored using docking and molecular simulations, revealing its safety for certain toxicities but potential risks for embryotoxicity and cardiac failure (Joshi and Srivastava, 2024).

This reviewed the cardiovascular protective effects of cinnamon, emphasizing its role in mitigating risks associated with diabetes and metabolic disorders due to compounds like cinnamaldehyde and cinnamic acid. Despite promising findings, further preclinical and clinical studies were recommended (Das *et al.*, 2022). The Investigated bioactive constituents of ginger (Zingiber officinale), including alkaloids, flavonoids, saponins, and tannins, attributing health benefits such as antimicrobial, anti-inflammatory, and cholesterol-lowering properties. Saponins, in particular, were noted for their ability to reduce cholesterol reabsorption (Umeoka, 2024). Developed sweet potato flakes enriched with chia seeds, demonstrating their cardioprotective potential in a rat model through improved lipid profiles and heart tissue preservation (Islam *et al.*, 2024).

Assessement the effects of various thermal treatments on the nutritional composition and flavonoid content of long-horned grasshoppers, Ruspolia differens. Blanching was found to enhance omega fatty acids and flavonoids, unlike deep frying, which reduced crude protein (Ochieng *et al.*, 2022). Phytochemical screening on spices like cinnamon, garlic, and turmeric, identifying carbohydrates, proteins, and bioactive compounds beneficial for health (Mulay and Salve, 2018). This study explored the ethnobotanical and pharmacological potential of alfalfa (Medicago sativa), noting its antioxidant and hypoglycemic properties and its role in phytoremediation (Savalia and Pandya, 2024). Phytochemical and toxicity studies on ginger extracts using GC-MS and LC-MS analysis. Acute toxicity tests confirmed the safety of ginger extracts at 5000 mg/kg in rats. Finally, an overview by researchers highlighted the antioxidant and anti-inflammatory potential of natural compounds such as resveratrol, berberine, and curcumin in treating heart diseases by targeting oxidative stress and inflammation (Zaki *et al.*, 2023).

Chia seeds (*Salvia hispanica L.*) have gained recognition as a "golden seed of the 21st century" due to their exceptional nutritional and nutraceutical benefits, including high omega-3 and omega-6 fatty acids, antioxidants, and essential amino acids. Studies highlighted their potential in genome editing to enhance these properties for improved health and industrial use (*The New Golden Seed for the 21st Century*, 2024). The bioactive compounds in pseudocereals, emphasizing their gluten-free and nutrient-rich profiles. These underutilized grains, containing saponins, polyphenols, and phytosterols, have potential for disease prevention and functional food development (Sánchez-Velázquez *et al.*, 2022). Explored associations between diet and health, focusing on bioactive compounds like peptides, omega-3, and fiber. These

compounds, found in cereals, pulses, fruits, and vegetables, have been linked to reduced risks of chronic diseases such as diabetes and cancer (Coelho *et al.*, 2022). Reviewed the bioactive protein and peptides derived from flaxseed meal, emphasizing their antioxidant, antibacterial, and ACE-inhibiting properties. While the health benefits are well-documented, challenges in large-scale production persist (Wu *et al.*, 2022). Provided insights into alpha-linolenic acid (ALA), a beneficial fatty acid found in plants, and discussed its limited conversion to EPA and DHA in the body. Research underscores ALA's role in improving stability, absorption, and its standalone benefits for nutritional product development (Zhu *et al.*, 2022). Explored bioactive peptides with multifunctional biological activities, such as antioxidative, anticancer, and antihypertensive effects. These peptides, derived from diverse food sources, are considered potential pharmaceutical ingredients, and bioinformatics tools play a critical role in advancing their study (Manzoor *et al.*, 2022). Highlighted the therapeutic potential of medicinal herbs (MH) in addressing cardiovascular diseases (CVDs) through phytotherapy. MH demonstrate anti-inflammatory and antioxidant properties, which improve dyslipidemia, atherosclerosis, and hypertension. However, large-scale clinical studies are needed to confirm these effects (Chávez-Castillo et al., 2022). Studied the impact of chia seeds on cardiac and hepatic protection in diet-induced obese rats, showing improved insulin sensitivity, reduced hepatic steatosis, and diminished cardiac inflammation through lipid redistribution (Poudyal *et al.*, 2022). a Systematic review and meta-analysis of 14 clinical trials on chia seeds, revealing their efficacy in reducing triglycerides, LDL cholesterol, and systolic blood pressure (Fateh *et al.*, 2024). Lastly, assessed the metabolic impact of chia seeds in obese prepubescent children, finding significant anti-inflammatory effects and correlations with reduced fibrinogen and tumor necrosis factor-α levels (da Silva *et al.*, 2024).

This investigated the anti-inflammatory and anti-atherosclerotic effects of chia seed protein and its fractions on macrophages. Simulated gastrointestinal digestion of chia protein reduced pro-inflammatory markers such as nitric oxide, reactive oxygen species, and cytokines like TNF-α, demonstrating potential in reducing inflammation and lipid accumulation in atherosclerosis (Grancieri *et al.*, 2022). Reviewed the bioactive compounds in *Acacia* species, identifying secondary metabolites such as flavonoids, alkaloids, and terpenes. These compounds exhibit anti-inflammatory, antidiabetic, and anticancer properties, emphasizing the need for further studies to isolate therapeutic agents (Batiha et al., 2022). Characterized bioactive compounds from *Acacia concinna* and *Citrus limon*, which showed antimicrobial and anticancer properties. Silver nanoparticles synthesized from *A. concinna* extract exhibited significant activity against pathogenic bacteria and MCF-7 cancer cells (Ahmed Shaikh *et al.*, 2022). Studied the effect of *Acacia concinna* extract on apoptosis in colon cancer cells, finding that it induced apoptosis via mitochondrial dysfunction and modulated intracellular pathways (Sitthisuk *et al.*, 2024). Examined plant extracts rich in tannins, saponins, and essential oils for their effects on rumen fermentation and conjugated linoleic acid

concentrations, highlighting their potential to improve feed efficiency and livestock health (Mandal *et al.*, 2016). Evaluated he antioxidant and anticancer activities of *A. concinna* leaves, using ORAC and FRAP assays for antioxidant capacity and a resazurin microplate assay for anticancer effects (Ganogpichayagrai and Suksaard, 2024).

Investigated the phytochemical profile and antioxidant potential of *Acacia concinna* extracts, finding significant tyrosinase inhibitory activity and antioxidant properties across different solvents (Rinthong *et al.,* 2024). Identified saponins in *A. concinna* pods as the bioactive compounds responsible for its anti-obesogenic activity. The compounds include acacic acid, monoterpenes, and sugars such as glucose and xylose (Zhuoyue *et al.*, 2021). Evaluated the anti-obesogenic potential of Thai medicinal plants, including *A. concinna*, using assays like pancreatic lipase inhibition and lipid accumulation suppression, confirming their efficacy in treating obesity (Ruangaram and Kato, 2020). Discussed the potential of phytosomes, lipophilic complexes derived from plant extracts, as novel drug delivery systems. These systems improve bioavailability and efficacy of bioactive compounds, addressing challenges in conventional drug delivery methods (Poudyal *et al.*, 2022). Reviewed the ethno-medicinal and pharmacological properties of *Bacopa monnieri*, emphasizing its antioxidant, anti-inflammatory, and neuroprotective effects. Its bioactive components show promise in treating neurological and inflammatory disorders (Kuruvalli *et al*., 2023). Finally, used computational methods to explore the potential of *Bacopa monnieri* phytochemicals as inhibitors of DPP-4 and PPAR-gamma for type-1 diabetes treatment (Susha and Sharma, 2021).

PREVIOUS WORK DONE IN THE RELEVANT AREA

The study titled "Molecular Docking Studies of Phytoconstituents Identified in Cinnamomum verum and Coriandrum sativum on HMG CoA Reductase – An Enzyme Target for Antihyperlipidemic Activity" investigates the potential of phytoconstituents from these medicinal plants to inhibit HMG CoA reductase, a critical enzyme in cholesterol biosynthesis. The research employs in-silico molecular docking techniques to evaluate the antihyperlipidemic activity of specific bioactive compounds compared to standard drugs like atorvastatin, pitavastatin, and simvastatin.

Obesity, a global health concern, is a significant risk factor for conditions like type-2 diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease. Although statins are widely prescribed for hyperlipidemia, their adverse effects (e.g., neuropathy, diabetes, and cognitive dysfunction) necessitate alternative treatments. Traditionally, plants like Cinnamomum verum and Coriandrum sativum have been used for their medicinal properties. This study aims to validate their antihyperlipidemic effects through reverse pharmacology and molecular docking.

The study used Molegro Virtual Docker (MVD) software to perform docking analyses of

phytoconstituents, such as cinnamaldehyde, eugenol, cinnamyl acetate, and geranyl acetate, identified from Cinnamomum verum and Coriandrum sativum. The 3D structure of the HMG CoA reductase enzyme (PDB ID: 3CCT) was obtained from the Protein Data Bank. The docking scores, rerank scores, and hydrogen bonding interactions of the phytoconstituents were compared with those of standard drugs.

Docking Scores: The MolDock scores, which reflect the potential binding affinity, showed that some phytoconstituents had promising inhibitory activity. Geranyl acetate and e-nerolidol demonstrated significant scores (-88.20 and -90.23, respectively) compared to standard drugs like atorvastatin (-182.68).

Hydrogen Bonding: The study analyzed hydrogen bond interactions, a critical parameter for stability. Cinnamic acid and eugenol exhibited notable hydrogen bond strengths, enhancing their binding potential to the enzyme. Drug-Likeness Properties: Using DruLiTo software, the phytoconstituents were evaluated for their drug-likeness properties based on Lipinski's Rule of Five. All phytoconstituents, except caryophyllene (due to high Log P value), demonstrated favorable pharmacokinetics.

The phytoconstituents from Cinnamomum verum and Coriandrum sativum exhibited inhibitory effects on HMG CoA reductase, suggesting their potential as natural antihyperlipidemic agents. These results justify the traditional claims of their use in managing hyperlipidemia and obesity-related disorders. Unlike synthetic statins, these compounds may offer fewer side effects, aligning with the concept of "food as medicine" proposed by Hippocrates. This study underscores the therapeutic potential of phytoconstituents in Cinnamomum verum and Coriandrum sativum for preventing hyperlipidemia. Their use as dietary supplements could mitigate cholesterol-related health risks naturally. Further research, including in-vivo studies and mechanism elucidation, is recommended to validate these findings and explore their clinical applications. The findings emphasize the role of phytoconstituents as safer, natural alternatives to synthetic drugs for hyperlipidaemia and highlight the importance of integrating traditional knowledge with modern pharmacological research ((Mavillapalli *et al.*, 2017).

LITERATURE REVIEW OF SOME NATURAL COMPOUNDS

The study explores natural compounds as alternatives to statins, which are widely used for cholesterol reduction but cause side effects like diabetes and myopathy. A virtual screening identified 22 natural compounds and 6 existing drugs with strong binding potential to HMG CoA reductase, the enzyme central to cholesterol synthesis. Pharmacokinetic and toxicity analyses indicated that natural compounds from commonly consumed foods could provide viable alternatives. Future experimental studies will validate these findings. Cholesterol, a critical biomolecule, is integral to cellular membrane structure and precursor biosynthesis for steroid hormones, bile acids, and vitamin D. Excess cholesterol (hypercholesterolemia) contributes to atherosclerosis, increasing cardiovascular risk.

Statins, current HMG CoA reductase inhibitors, lower LDL cholesterol but have side effects, necessitating

natural alternatives. Natural products (secondary metabolites) offer structural diversity, enabling effective interaction with target proteins. In silico methods enhance efficiency in natural product drug discovery. Target: Human HMG CoA reductase, a rate-limiting enzyme in cholesterol synthesis. Structural data was retrieved from the Protein Data Bank (PDB ID: 1HW8).

Ligands: Compounds were sourced from PhytoHub, a database for dietary phytochemicals. Additionally, virtual screening using MTiOpenScreen identified 183 compounds, including natural products and synthetic drugs. Toxicity and Pharmacokinetics Analysis Osiris software evaluated toxicity, filtering out mutagenic or harmful compounds. SWISSADME assessed physicochemical properties (Lipinski's rule, hydrogen bond donors/acceptors, molecular weight, etc.), identifying 114 safe compounds. Binding and Interaction Analysis Docking simulations in Discovery Studio identified binding affinities and interactions between ligands and HMG CoA reductase. Compounds were evaluated for binding energy, hydrogen bond formation, and non-covalent interactions.

Natural Compounds Identified A total of 558 phytochemicals from 45 foods (e.g., spices, citrus fruits) were analyzed. Notable foods included black cumin, cardamom, cinnamon, ginseng, ginger, kale, green tea, and tomatoes. 46 natural compounds displayed strong drug-like properties (e.g., luteolin, curcumin, phloretin, hesperetin, epicatechin). Pharmacokinetics and Toxicity Compounds like curcumin, phloretin, and catechin adhered to Lipinski's rule of five and showed favorable ADME profiles.

Toxicity analysis confirmed non-mutagenicity and biological safety for the final 46 compounds.

Binding Interactions Docking revealed strong interactions with the active site of HMG CoA reductase, including amino acids like Cys561, Ser565, Asn755, Glu559, Lys691, and His752.

Curcumin demonstrated the highest dock score (118.849), forming multiple hydrogen bonds with residues like Ser684, Lys735, and Arg590. Statistical Highlights 73 compounds progressed to advanced analysis, with 46 being natural and 27 synthetic. Docking studies validated the potential inhibitory activity of these compounds, confirming their ability to mimic statin binding patterns.

The findings support natural compounds as promising HMG CoA reductase inhibitors with fewer side effects than synthetic statins. Compounds like phloretin, luteolin, and curcumin exhibited potential for experimental and clinical evaluation. Future *in vitro* and *in vivo* studies will confirm enzymatic inhibition and therapeutic viability. The study highlights computational methods as efficient tools for identifying natural compounds with therapeutic potential. It advocates for integrating food-based bioactives into cholesterol management strategies, emphasizing a reduced reliance on synthetic statins.

NEED OF STUDY

The rising prevalence of atherosclerosis, obesity, dyslipidemia and cardiovascular coupled with the limitations of existing treatments, underscores the need for innovative and integrative approaches to

disease management. phytoconstituents are widely recognized for their health benefits, their mechanisms of action and therapeutic potential in the context of atherosclerosis, obesity, dyslipidemia and cardiovascular require further elucidation. The study focuses on the need for in vivo evaluations, which are essential for understanding the pharmacodynamics, toxicity, and long-term safety of these phytochemicals. Experimental animal models provide a controlled environment to assess these parameters, paving the way for clinical trials. Present work aims to optimize the use of phytochemicals through advancements in formulation science. By reducing reliance on synthetic drugs and promoting sustainable practices, this research aligns with the broader goals of public health and environmental conservation. The phytoconstituents studies are attempted but not fully explored so this present work needs to asses systematic *In vitro* and *In vivo* studies.

OBJECTIVES OF PRESENT STUDY

Aim:

To explore and evaluate the potential of selected phytoconstituents of spices used in Indian cuisine in mitigating obesity and atherosclerosis,/dyslipidemia, utilizing network pharmacology, molecular docking, *in vitro* and *in vivo* methodologies to identify their therapeutic efficacy and mechanisms of action.

Objectives:

1. **Selection of Phytoconstituents**:

Identify and select bioactive compounds from commonly used Indian spices (such as curcumin from turmeric, piperine from black pepper, and other bioactive molecules from spices like cumin, cinnamon, etc.) with reported anti-inflammatory, antioxidant, and lipid-lowering properties.

2. **Network Pharmacology Analysis**:

Construct a network pharmacology model to predict the multi-target effects of selected phytoconstituents on obesity, atherosclerosis, and dyslipidemia.

Identify key biological targets (e.g., receptors, enzymes) and pathways (e.g., lipid metabolism, inflammation, adipogenesis) involved in the therapeutic effects of these compounds.

Use bioinformatics tools to build a molecular interaction network that integrates information from databases like PubChem, KEGG, and Gene Ontology (GO).

3. **Molecular Docking Studies**:

Perform molecular docking simulations to evaluate the binding affinity of selected phytoconstituents to key targets associated with obesity, atherosclerosis, and dyslipidemia (e.g., PPAR-γ, LDL receptor, SREBP-1c).

Analyze the interaction modes and predict the inhibitory or activating effects of the compounds on these molecular targets.

4. ***In Vitro* Assessment**:

Conduct cell-based assays (e.g., 3T3-L1 adipocyte differentiation, macrophage polarization) to evaluate the anti-obesogenic and anti-inflammatory effects of the selected phytoconstituents.

Assess their impact on lipid accumulation, oxidative stress, and inflammatory cytokine release in relevant cell models.

5. ***In Vivo* Assessment**:

Investigate the anti-obesogenic, anti-atherosclerotic, and anti-dyslipidemic effects of selected phytoconstituents using animal models (e.g., high-fat diet-induced obesity and atherosclerosis models).
Measure key biomarkers such as body weight, lipid profile, glucose metabolism, inflammatory cytokines, and histopathological changes in tissues (liver, adipose, and arterial tissues).

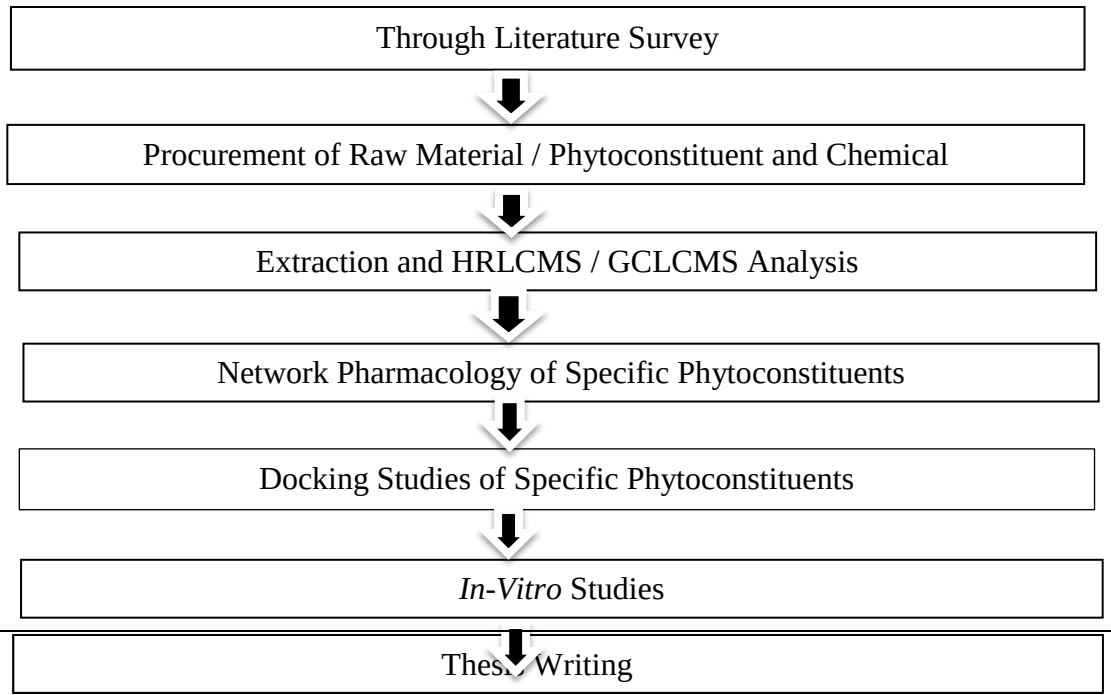
6. **Comparative Analysis and Potential Synergistic Effects**:

Compare the effects of individual phytoconstituents and their potential synergistic interactions in combination, to identify more effective formulations or combinations for treating obesity, atherosclerosis, and dyslipidemia.

7. **Validation and Clinical Relevance**:

Correlate the experimental results with clinical data or published clinical studies to assess the relevance and potential for human application of the phytoconstituents in managing obesity, atherosclerosis, and dyslipidemia.

PLAN OF WORK


Through Literature Survey
Procurement of Raw Material / Phytoconstituent and Chemical
Extraction and HRLCMS / GCLCMS Analysis
Network Pharmacology of Specific Phytoconstituents
Docking Studies of Specific Phytoconstituents
In-Vitro Studies
Thesis Writing

METHODOLOGY TO BE ADOPTED

1. Selection of Indian Spices and Phytoconstituents

Objective: Choose relevant spices based on their traditional use in treating metabolic disorders such as obesity, atherosclerosis, and dyslipidemia.

Spices Selection Criteria: Commonly used Indian spices like turmeric (Curcuma longa), garlic (Allium sativum), ginger (Zingiber officinale), cinnamon (Cinnamomum verum), fenugreek (Trigonella foenum-graecum), etc.

Phytoconstituents Selection: Focus on bioactive compounds from these spices, such as curcumin, allicin, gingerol, cinnamaldehyde, and saponins. Information can be gathered from literature reviews, databases like PubMed, and phytochemical databases like PubChem.

2. Network Pharmacology Approach

Objective: Predict the potential biological targets and pathways involved in anti-obesity, anti-atherosclerosis, and anti-dyslipidemia mechanisms.

Tools and Databases:

Target Identification: Use network pharmacology tools (e.g., STITCH, STRING, PharmMapper) to identify target proteins associated with obesity, atherosclerosis, and dyslipidemia.

Gene Ontology (GO) Analysis: Identify the molecular function, biological process, and cellular component related to the target proteins.

Pathway Enrichment Analysis: Use KEGG and Reactome pathways to analyze the pathways affected by the identified targets.

Integration of Information: Combine the phytoconstituents with the identified molecular targets and pathways using network pharmacology platforms like Cytoscape to create a comprehensive pharmacological network.

3. Molecular Docking Studies

Objective: Predict the binding affinity and mode of interaction between the phytoconstituents and their target proteins.

Tools:

Protein Preparation: Obtain 3D structures of target proteins from PDB (Protein Data Bank) or model them using homology modeling if needed (using tools like Modeller).

Ligand Preparation: Collect the 3D structures of the phytoconstituents from PubChem or ChemSpider and prepare them for docking.

Docking Simulation: Perform molecular docking using tools like AutoDock Vina, Docking Server, or GOLD to analyze the binding affinity and interactions between the phytoconstituents and the target proteins.

Analysis: Evaluate the docking results by looking at binding energy, binding site interactions, and possible mechanisms of action.

4. *In Vitro* Evaluation

Objective: Experimentally validate the effect of selected phytoconstituents on cellular models related to obesity, atherosclerosis, and dyslipidemia.

Cell Lines Selection:

Adipogenesis Models (Obesity): Use 3T3-L1 preadipocytes or human adipocytes to assess the effect on adipogenesis and lipogenesis.

Atherosclerosis Models: Use human endothelial cells (HUVEC) or vascular smooth muscle cells (VSMC) to study inflammation and endothelial dysfunction.

Dyslipidemia Models: HepG2 cells or macrophages can be used to study lipid metabolism and lipid accumulation.

Experimental Setup:

Treatment with Phytoconstituents: Expose the cell lines to varying concentrations of phytoconstituents (based on published IC50 values or preliminary dose-response studies).

Assays: Perform assays to assess lipid accumulation (e.g., Oil Red O staining for fat accumulation), inflammation markers (e.g., IL-6, TNF-α), and lipid profile (e.g., LDL, HDL, TG levels).

Gene Expression Studies: Use qPCR and Western blotting to assess the expression of key genes (e.g., PPAR-γ, SREBP-1, ADIPOQ) involved in obesity and atherosclerosis.

5. *In Vivo* Evaluation

Objective: Validate the effects of selected phytoconstituents in animal models of obesity, atherosclerosis, and dyslipidemia.

Animal Model Selection:

Obesity: Use high-fat diet (HFD)-induced obese rats or mice to assess weight gain, adiposity, and metabolic parameters.

Atherosclerosis: Use Apolipoprotein E (ApoE) knockout mice or Apolipoprotein B (ApoB) transgenic mice to study the progression of atherosclerotic plaques.

Dyslipidemia: Use diet-induced dyslipidemia models (e.g., high-fat or high-cholesterol diet).

Treatment Protocol:

Administer phytoconstituents orally or via injection to the animals for a specific period (e.g., 4-8 weeks).

Control group with vehicle (e.g., saline) for comparison.

Parameters to Evaluate:

Body weight, food intake, and adiposity index.

Lipid profile: Measure serum levels of cholesterol, triglycerides (TG), HDL, LDL, and VLDL.

Inflammatory markers: Assess markers like C-reactive protein (CRP), TNF-α, and IL-6 in serum.
Histopathological studies: Examine tissues like liver (for fatty liver), aorta (for atherosclerotic plaques), and adipose tissue (for adipogenesis) using H&E staining and Oil Red O staining.
Biomolecular Mechanism: Measure the expression of key proteins involved in lipid metabolism, inflammation, and adipogenesis using Western blotting or Immunohistochemistry.

6. Statistical Analysis

Objective: Analyze the data obtained from in vitro and in vivo experiments to determine the significance of findings.
Statistical Tools: Use GraphPad Prism or SPSS for statistical analysis.
Comparison between groups: Perform ANOVA followed by post-hoc tests (e.g., Tukey's test) for comparisons between control and experimental groups.
Significance: Set the p-value threshold at < 0.05 for statistical significance.

REFERENCES

1. WHO. (2022). Cardiovascular diseases. Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases
2. Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., & Kajo, K. (2021). Phytochemicals targeting oxidative stress and inflammation in atherosclerosis: Potential therapeutic applications. Antioxidants, 10(5), 716.
3. Patel, P., Thakkar, J. H., & Patel, V. (2020). Evaluation of antihypertensive activity of Boerhaavia diffusa. Journal of Ethnopharmacology, 253, 112146.
4. NFHS-5. (2021). National Family Health Survey (NFHS-5), Ministry of Health and Family Welfare, India. Available at: https://rchiips.org/nfhs/nfhs-5.shtml
5. OECD. (2020). The Heavy Burden of Obesity: The Economics of Prevention. Available at: https://www.oecd.org/health/the-heavy-burden-of-obesity-67450d67-en.htm
6. Jin, S., & Cho, K.-H. (2011). Water extracts of cinnamon and clove exhibit potent inhibition of protein glycation and anti-atherosclerotic activity in vitro and in vivo hypolipidemic activity in zebrafish. *Food and Chemical Toxicology, Elsevier*
7. Joshi, S., & Srivastava, R. (2024). Tracing the pathways and mechanisms involved in medicinal uses of flaxseed with computational methods and bioinformatics tools. *Frontiers in Chemistry*.
8. Das, G., Gonçalves, S., Heredia, J. B., Romano, A., Jiménez-Ortega, L. A., Gutiérrez-Grijalva, E. P., Shin, H. S., & Patra, J. K. (2022). Cardiovascular protective effect of cinnamon and its major bioactive constituents: An update. *Journal of Functional Foods, Elsevier*.

9. Umeoka, N. (2024). Phytochemical analysis of ginger rhizome extract. *Journal of Health, Wellness & Safety Research*.
10. Islam, D., Akter, F., Akhter, S., Siddique, A., Lipy, E. P., Hakim, M., Paula, T., Chowdhury, F., Hossain, H., & Hossain, S. (2024). Characterization of sweet potato flakes enriched with chia seeds: Nutritional profile, bioactive compounds, sensory attributes, and cardioprotective potential.
11. Ochieng, B. O., Anyango, J. O., Nduko, J. M., Cheseto, X., Mudalungu, C. M., Khamis, F. M., Ghemoh, C. J., Egonyu, P. J., Subramanian, S., Nakimbugwe, D., & Ssepuuya, G. (2022). Dynamics in nutrients, sterols, and total flavonoid content during processing of the edible long-horned grasshopper (Ruspolia differens Serville) for food. *Elsevier*.
12. Mulay, J. R., & Salve, J. (2018). Phytochemical analysis of some selected spices. *IJEDR*.
13. Savalia, V., & Pandya, D. (2024). Traditional, ethnobotanical, phytopharmacological, and nutraceutical potential of Medicago sativa Linn. *African Journals of Biological Sciences*.
14. Zaki, M. M., Elhassan, A. E. A., El Zahid, E. S. M., A., M. K. A., Hejab, A. M. I., Kamel, A. H.,
15. Amin, T. R., & Hassan, S. S. M. (2023). Phytochemical screening and toxicity studies for ginger extracts with evaluation of some biochemical parameters and anticoagulant bioactivity. *Egyptian Journal of Chemistry*.
16. *Bioactive lead compounds and molecular targets for the treatment of heart diseases* (2023).
17. *The New Golden Seed for the 21st Century: Nutraceutical Properties and Technological Uses* (2024).
18. Sánchez-Velázquez, O. A., Manzanilla-Valdez, M. L., Mondor, M., Melgar-Lalanne, G., & Hernández-Álvarez, A. J. (2022). Ancient grains as sources of bioactive compounds for functional foods and modulation of molecular markers. *Ancient Grains as Sources of Bioactive Compounds*.
19. Coelho, M. S., Fernandes, S. S., & Salas-Mellado, M. M. (2022). Association between diet, health, and the presence of bioactive compounds in foods.
20. Wu, S., Wang, X., Qi, W., & Guo, Q. (2022). Bioactive protein/peptides of flaxseed: A review.
21. Zhu, P., Fan, L., Yan, X., & Li, J. (2022). Advances of α-linolenic acid: Sources, extraction, biological activity, and its carrier.
22. Manzoor, M., Singh, J., & Gani, A. (2022). Exploration of bioactive peptides from various origins as promising nutraceutical treasures: *In vitro*, *in silico*, and *in vivo* studies.
23. Chávez-Castillo, M., Ortega, Á., Durán, P., Pirela, D., Marquina, M., Cano, C., Salazar, J., Gonzalez, M. C., Bermúdez, V., Rojas-Quintero, J., & Velasco, M. (2022). Phytotherapy for cardiovascular disease: A bench-to-bedside approach.

24. Poudyal, H., Panchal, S. K., Waanders, J., Ward, L., & Brown, L. (2022). Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats.
25. Fateh, H. L., Ahmed, D. H., Najafabadi, M. S., & Moludi, J. (2024). The impact of chia seeds on diabetes, blood pressure, lipid profile, and obesity indicators: Systematic review and meta-regression analysis of 14 randomized controlled trials.
26. da Silva, C. S., Monteiro, C. R. A., da Silva, G. H. F., Sarni, R. O. S., Suano Souza, F. I., Feder, D., & Messias, M. C. F. (2024). Assessing the metabolic impact of ground chia seed in overweight and obese prepubescent children: Results of a double-blind randomized clinical trial.
27. Grancieri, M., Martino, H. S. D., & Mejia, E. G. D. (2022). Chia (Salvia hispanica L.) seed total protein and protein fractions digests reduce biomarkers of inflammation and atherosclerosis in macrophages *in vitro*.
28. Batiha, G. E. S., Akhtar, N., Alsayegh, A. A., Abusudah, W. F., Almohmadi, N. H., Shaheen, H. M., Singh, T. G., & De Waard, M. (2022). Bioactive compounds, pharmacological actions, and pharmacokinetics of genus *Acacia*. *MDPI*.
29. Ahmed Shaikh, I., Muddapur, U. M., Bagewadi, Z. K., Chiniwal, S., Ghoneim, M. M., Mahnashi, M. H., Alsaikhan, F., et al. (2022). Characterization of bioactive compounds from *Acacia concinna* and *Citrus limon*, silver nanoparticles' production by *A. concinna* extract, and their biological properties.
30. Sitthisuk, P., Innajak, S., Poorahong, W., Samosorn, S., Dolsophon, K., & Watanapokasin, R. (2024). Effect of *Acacia concinna* extract on apoptosis induction associated with endoplasmic reticulum stress and modulated intracellular signaling pathway in human colon HCT116 cancer cells.
31. Mandal, G. P., Roy, A., & Patra, A. K. (2016). Effects of plant extracts rich in tannins, saponins, and essential oils on rumen fermentation and conjugated linoleic acid concentrations *in vitro*. *Indian Journal of Animal Health*.
32. Ganogpichayagrai, A., & Suksaard, C. (2024). Evaluation of proximate composition and biological activities of *Acacia concinna* leaves in Thailand.
33. Rinthong, P.-O., Katisart, T., & Maneechai, S. (2024). Evaluation of the phytochemical profiling, tyrosinase inhibitory activity, and antioxidant potential of various extracts of *Acacia concinna* (*Willd.*) DC.
34. Zhuoyue, Z., & Ruangaram, W. (2021). Saponins are responsible for the anti-obesogenic activity of *Acacia concinna*. *Journal of Natural Medicines*.

35. Ruangaram, W., & Kato, E. (2020). Selection of Thai medicinal plants with anti-obesogenic potential via in vitro methods. *MDPI*.
36. Poudyal, A., Subba, B., Bhattarai, A., Regmi, D., Tiwari, B., Pradhan, A., & Bhutia, S. (2022). A comprehensive review on phytosomes: A novel drug delivery system of phytoconstituents. *International Journal of Life Science and Pharma Research*.
37. Kuruvalli, G., Wankhade, I., Wankhede, S., Budiyal Ramesh, S., Narayanaswamy, C. K., Munikrishnappa, G. N., & Reddy, V. D. (2023). A comprehensive review on the ethno-medicinal and pharmacological properties of *Bacopa monnieri*.
38. Susha, D., & Sharma, S. (2021). A computational approach of phytochemicals from *Bacopa monnieri* in contrast to DPP-4 and peroxisome proliferator-activated receptors gamma as a target for type-1 diabetes. *World Journal of Biological and Pharmaceutical Research*.

Dr. DHAWALE S.C.
Ph.D. Supervisor

Ms. MANE .M.S.
Research Student